# Kongeriget Danmark

Patent application No.: PA 2001 00285

Date of filing: 21 February 2001

Applicant: Novozymes A/S
Krogshøjvej 36
DK-2880 Bagsværd

This is to certify the correctness of the following information:

The attached photocopy is a true copy of the following document:

- The specification, claims and abstract as filed with the application on the filing date indicated above.

Patent- og Varemærkestyrelsen
Kongeriget Danmark

Patent- og
Varemærkestyrelsen
Erhvervsministeriet

TAASTRUP 01 March 2001

Karin Schlichting
Head Clerk

27

NZAS-0013561

# PRODUCTION OF FRIED PRODUCTS

## FIELD OF THE INVENTION

The invention relates to a process for manufacturing fried starchy products, in particular fried instant noodles and the products produced by such process.

## BACKGROUND OF THE INVENTION

Instant noodles have widespread consumer support for their superior instant cooking characteristics. Instant noodles can very easily be made ready to eat by merely pouring boiling water onto them in a dish or by boiling them for only a short time. Instant noodles are also highly regarded for their characteristics as a staple food and good storage (or keeping) quality.

Instant noodles are classified according to the drying method into (i) a fried noodle (fried type), (ii) a hot air-dried noodle (non-fried type) and a (iii) freeze-dried noodle (freeze-dried type). The fried types, are of interest since they are very good with respect to instant cooking, ease of handling, ease of manufacture and keeping quality.

Several different methods for producing fried instant noodles have been disclosed. In general, there is a demand for improved processes for the manufacturing of fried instant noodles. One concern in respect of fried instant noodles is that they absorb a large amount of oil when fried. Since, in recent years, there has been strong consumer demand for food products with low fat content, a method for producing fried instant noodles with low oil absorption is also desirable.

Several enzymes have been disclosed for use in manufacturing of noodles. For example, WO 9844804 discloses use of glycerol oxidase, optionally together with lipase, to produce dough for bread, pasta or noodles. EP 575 133 A2 discloses a phospholipase A1 from *Aspergillus* and mentions that flour treated with the enzyme may be used for making noodles. US 5,916,619 relates to a method for producing fried instant noodles and discloses a method wherein noodle strands containing a chemical leavening agent and at least one enzyme selected from the group consisting of amylase and protease are steamed and fried, which method is disclosed as lowering the oil absorption of the fried instant noodles. US 4,567,046 mentions treating cereal flour dough with a phospholipase A to make noodles.

Use of a phospholipase has also been disclosed for making baked products, e.g. US 4,567,046 and EP 171,995 A disclose that addition of phospholipase A to dough enhances retardation of staling of bread made from such a dough. WO

99/53769 discloses a process for preparing bread comprising adding to the dough an anti-staling amylase and a phospholipase for improving the softness of the bread and retarding the staling of the bread after baking. Further EP 1057415 A1 discloses use of a lipase in a process for making pasta.

None of these documents disclose use of an enzyme having phospholipase activity for making a fried starchy product, including fried instant noodles, or the benefit of doing so.

## SUMMARY OF THE INVENTION

The inventor has found that treatment with an enzyme having phospholipase activity improves several properties of fried instant noodles. Of particular value is that the inventor have found that the oil content after frying is reduced and that mouthfeel and appearance are improved. Accordingly, fried instant noodles made according to the process of the invention showed improvements in several parameters: increased surface firmness, increased core firmness, increased surface smoothness, improved mouthfeel, improved cooked noodle appearance and reduced oil content of fried noodles.

Thus, the invention provides a process for preparing a fried product comprising incorporation of an enzyme having phospholipase activity into a dough and then frying to obtain a fried product, in particular a fried starchy product. The invention also provides such fried products, including fried instant noodles and snack products, prepared by a process of the invention and the use of an enzyme having phospholipase activity in a process of the invention, in particular for reducing the oil content of the fried product so produced. The invention further provides use of an enzyme having phospholipase activity to make a pre-mix comprising flour, preferably wheat flour, and said enzyme, which pre-mix is to be used in a process for making a (starchy) fried product, including, e.g., fried instant noodles.

## DETAILED DESCRIPTION OF THE INVENTION

The invention provides an alternative process for producing fried (starchy) products, in particular fried instant noodles. One object of the invention is to provide fried instant noodles having improved properties, e.g. a lower oil content after frying and/or improved texture. As shown in the examples the inventor has found that treatment with an enzyme having phospholipase activity improves several properties

of fried instant noodles, including a reduction of the oil content after frying and improvement of texture properties.

Accordingly, the invention relates to a process for preparing a fried product comprising incorporating into a dough an enzyme having phospholipase activity. The process of the invention comprises the steps of: (i) incorporation of an enzyme having phospholipase activity into a dough; and (ii) frying to obtain a fried product. Depending on the type of product to be produced, the process may also comprise the step of shaping the dough into a desired form prior to frying.

The process of the invention for manufacturing a fried product may comprise, in addition to the treatment with the enzyme having phospholipase activity, any process steps desired for producing the fried product in question. Thus, the process of the invention for manufacturing fried instant noodles may comprise, in addition to the treatment with the enzyme having phospholipase activity, any process steps desired for the production of fried instant noodles.

The process of the invention may e.g. comprise the step of steaming prior to frying. With respect to fried instant noodles, the steaming, if done, is preferably performed on noodle strands prior to frying, but one may also steam noodle sheets before dividing into noodle strands.

As an example the following methods may be used to make the fried instant noodles (1) kneading raw materials, preferably comprising cereal flour, for noodles to make a dough, the dough is compounded and rolled into a sheet which is cut into noodle strands; and (2) an extrusion method wherein raw materials for noodles are kneaded in the same manner as for pasta and the resulting dough is extruded into noodle strands using an extruder.

The noodle strands may also be produced by other methods than the above methods (1) and (2), e.g., a manual rolling method, a vacuum extrusion method or the like. No limitation is made in the present invention as to the processes and conditions for kneading; for compounding and rolling; for cutting the noodle strands; and for extruding the noodle strands or as to the type of equipment used for those processes.

Accordingly, the mixed dough may be compressed between steel rollers or otherwise compressed to form a discrete dough sheet of the desired width. Preferably, the dough sheet is then sequentially reduced in thickness, e.g. by successive passes through steel rollers with varying roller gaps. When the desired thickness is reached the dough is preferably slit lengthways into strips. The dough

may be rested after mixing, or during the sheeting process, to allow chemical (polymeric) structures to relax and further chemical reactions to take place.

The thickness, width, diameter and the like of the noodle strand can be adjusted depending on the type of fried instant noodles. In general, a noodle strand with a thickness of about 0.7 to 1.6 mm and a width of about 1.0 to 2.0 mm, or a noodle strand with a diameter of about 0.9 to 1.5 mm is preferred.

The noodle strands are preferably subjected to a steaming, e.g. in the range of 95-100°C. The noodle strands may e.g. be in a raw state or half-dried before steaming. It is preferred that the enzyme(s) used in the process of the invention is mixed uniformly in the noodle dough. Any process and apparatus suitable for steaming of instant noodles may be employed. For example, the noodle strands may be processed with steam at a temperature of 95°C to 100°C while continuously transferring the noodle strands on a net conveyor or the like, or by packing the noodle strands in a basket or the like and placing, batch-wise, into a steam chamber. Steaming is done for any period found suitable, e.g. for a period of time from e.g. 30 second up to, but not limited to, 5 min. Steaming may be conducted e.g. at atmospheric pressures or higher as required. The term steaming as used herein also encompasses treatment in a microwave oven or other methods to obtain a steaming-like result, depending on the circumstances and the products produced.

Subsequently, the noodles (preferably steamed) are fried by any suitable method. For example, each single serving is filled into a container, a frame or the like to provide one package of noodles, and the single package of noodles is fried and dehydrated to manufacture the fried instant noodles. This frying process is not limited. Generally, edible oils such as palm oil, partially hydrogenated palm oil, refined palm oil, pure lard, modified lard, and mixtures of these are used. The noodle strands are fried for e.g. about ½ to 3 minutes at temperatures of about 130-170°C as desired by the manufacturer.

The resulting fried noodles may be cooled, packed, cartoned and/or encased in any suitable manner and then stored, distributed and sold as the case may be. In preparation for consumption, the noodles are either simply rehydrated for a specified time in very hot water or are boiled for a specified time which is dependent on the cross-sectional area of the noodle strands. The fried instant noodles of the present invention may be flavoured and seasoned, or alternatively accompanied by a packet of soup.

The dough in the process of the invention is preferably made from flour from a starchy raw material, i.e. raw material suitable for making a dough. The term dough as used herein encompasses batter as the case may be. The term starchy in the context of "starchy product" and starchy raw material, means that it comprises starch, preferably as the major single component calculated on basis of dry matter; "single" component means that compared to each of the other ingredients taken alone, starch is present in a higher amount on a weight to weight basis.

Thus, the invention relates to a process for preparing a fried product comprising incorporation of an enzyme having phospholipase activity into a dough and then frying to obtain a fried product, such product may also be termed a fried starchy product. The dough in a particularly interesting embodiment comprises flour from cereals, i.e. the invention also includes a process as disclosed herein for providing fried cereal based products, also termed "fried cereal products". The dough in the process of the invention, preferably comprises flour from a cereal and/or from an other plant source. The plant source, e.g. cereals, used to make the dough, may be of any type and origin suitable for making the fried product in question, including e.g. fried instant noodles or snack products. The cereal may be selected from the group consisting of, but not limited to, wheat, rye, barley, oats, maize/corn, rice, sorghum, millet, and buckwheat, as well as any mixtures thereof. The other plant source may be selected from the group consisting of, but not limited to, potato, sweet potato, yam, taro, tapioca, and beans, such as, e.g., soy bean and mung bean.

Accordingly, any cereal flour suitable in the manufacture of noodles can be used, examples of which include wheat flour, durum wheat flour, rye flour, soybean flour, oat flour, buckwheat flour, rice flour; starches such as potato starch, tapioca starch, corn starch and the like; the alpha converted form of these cereal flours; dogtooth violet starch; yam starch and the like. These cereal flours may be used alone or in admixture therewith. The cereal flour may be selected depending on the variety of noodle to be manufactured. For fried instant noodles, the dough in a preferred embodiment of the invention, is made from cereals, preferably wheat flour.

The present inventor has found that by treatment with the enzyme having phospholipase activity, the resulting fried instant noodles (whether made from so-called low or high quality wheat, i.e. with a low or high protein content, respectively) have a decreased oil content. Fried instant noodles made from wheat flour with a low protein content generally have a higher oil content than fried instant noodles made from wheat flour with a high protein content [Moss R., Gore P.J., Murray I.C. 1987.

The influence of ingredients and processing variables on the quality and microstructure of Hokkien, Cantonese and instant noodles. Food Microstructure 6: 63 – 74. and Kim. S.-K., 1996. Instant noodles pp 195 – 225 in: Pasta and Noodle Technology. J.E. Kruger, R.B. Matsuo and J.W. Dick eds. American Association of Cereal Chemists Press St Paul MN USA]. Thus, the inventor has provided a process to improve the quality of fried instant noodles made from wheat flour having a relatively low content of protein.

In further embodiments, in the process of the invention, preferably for making fried instant noodles, the dough is made from a cereal flour being substantially only wheat flour, which wheat flour comprises less than 15%, e.g. less than 12%, such as less 10% (w/w) protein. However, the protein content of the flour used to make the dough in the process of the invention, may be of any amount which is found suitable for making the fried product in question, i.e. e.g. also having a protein content higher than 10%, such as in the range of 10-30% (w/w).

In one embodiment, in the process of the invention the dough is a noodle dough and the fried product is fried instant noodle.

The process of the invention may comprises the steps of (i) preparing a noodle dough comprising cereal flour (e.g. wheat flour), water and an enzyme having phospholipase activity; (ii) making noodle strands from the noodle dough; and (iii) frying the noodle strands; optionally steaming the noodle strands prior to frying, either prior to or after step (ii). Included is also a process for manufacturing fried instant noodles wherein noodle strands comprising cereal flour (e.g. wheat flour), and an enzyme having phospholipase activity are fried, optionally steaming the noodle strands before frying.

To produce fried instant noodles by the process of the invention, the noodle dough preferably comprises cereal flour, water and said enzyme having phospholipase activity. In a preferred embodiment, the flour is wheat flour. The noodle dough may further comprise one or more ingredients selected from the group consisting of a chemical leavening agent, NaCl, kansui (e.g., but not limited to, sodium carbonate, potassium carbonate or a mixture of these or other salts).

In further embodiments, the content of sodium carbonate, potassium carbonate, sodium hydroxide individually or mixtures of any or all of these salts, if present at all, in the dough in the process of the invention is at most 1.5% (w/w) - based on flour, e.g. at most 1% (w/w), or at most 0,5% (w/w), such as e.g. in the range of 0.1-1.5% (w/w), preferably 0.1-0.5%(w/w), such as about 0.3%(w/w).

The dough may also comprise one or more of the ingredients selected from the group consisting of guar gum, locust bean gum, alginates, xanthan gum, carboxymethyl-cellulose, methyl-cellulose, monoglycerides, diacetyltartaric esters of mono-and di-glycerides (DATEM), wheat gluten, phosphate salts, or native starches from botanical sources other than the primary cereal flour used to make the dough, or chemically or physically modified starches from any botanical source which could include one or more starches selected from, but not limited to, the group hydroxypropyl starches or cross-linked starches or partly hydrolyzed starches or cold-water swelling starches.

The dough may also comprise one or more additives such as emulsifying agents, protein enhancing agents, polysaccharides for increasing viscosity, oligosaccharides, polymerized phosphates, coloring materials, nutrition enhancing agents, powdered chlorella, powdered skim milk, powdered vegetables, powdered seaweeds, antioxidants, whole egg, egg white and the like.

Examples of chemical leavening agent which may be used in the process of the invention, is at least one gas generating agent such as sodium hydrogen carbonate, ammonium carbonate, ammonium dicarbonate, potassium carbonate, and the like; and a mixture of the such gas generating agents with one or more gas generating promotion agents such as tartaric acid, potassium hydrogen tartrate, fumaric acid, sodium fumarate, glucono delta-lactone, calcium primary phosphate, sodium phosphate, anhydrous ammonium aluminiumchloride (burnt ammonium alum), anhydrous potassium aluminium sulphate (burnt alum), disodium dihydrogen pyrophosphate and the like. Any chemical leavening agent may be added to the formulation in an amount found suitable. In other embodiments of the invention, the dough, in particular the noodle dough, does not comprise a chemical leavening agent.

The enzyme having phospholipase activity may act on phospholipid provided by cereal flour in the dough. However, the desired effect may be increased by adding a phospholipid, e.g. in an amount of 0.05-20 g/kg of flour, e.g. 0.1-10 g/kg. The phospholipid may be a diacyl-glycero-phospholipid, such as lecithin or cephalin. Thus, the process may further comprise incorporating a phospholipid (e.g. lecithin) into the dough, e.g. it being a noodle dough. The process of the invention, may also comprise addition of fat to the dough. However, in other embodiments of the invention, fat is not added to the dough. The process of the invention may comprise addition of one or more emulsifiers to the dough, e.g. monoglycerides and

diacetyltartaric esters of mono-and di-glycerides. In another embodiment, the process of the invention does not comprise addition of emulsifiers other than the phospholipid. In other embodiments of the invention, phospholipids are not added to the dough.

The process of the invention may also comprise addition to the dough of one or more additional enzymes not being enzymes having phospholipase activity, such as e.g. an amylase and/or a protease.

**Enzyme having phospholipase activity**

The enzyme having phospholipase activity used in the process of the invention may be from any source and may be derived or obtained from any organism, including the organism as disclosed herein. Accordingly, the enzyme having phospholipase activity may have an amino acid sequence corresponding to a wildtype sequence (obtainable from a natural source) or it may be a variant having one or more amino acid residues which have been modified (having one or more amino acids which are deleted, inserted and/or substituted) compared to a wildtype sequence, e.g. produced by any suitable recombinant techniques or shuffling, provided that (as indicated by the term "enzyme having phospholipase activity") the enzyme has phospholipase activity. The term "derived from" includes in this context both wildtype and variants thereof, whereas the termed "obtainable from" is used to denote only wildtype enzyme (i.e. native enzymes). Within the meaning of native enzyme are included natural variants, e.g. allelic variants. The term "derived" also encompasses enzymes which have been modified e.g. by glycosylation, phosphorylation etc., whether in vivo or in vitro.

As indicated above, the term "obtainable" in this context means that the enzyme has an amino acid sequence identical to a native enzyme. The term encompasses an enzyme that has been isolated from an organism where it is present natively, or by any other method. In the context of recombinantly produced enzymes the terms "obtainable" and "derived" refer to the identity of the enzyme and not the identity of the host organism in which it is produced recombinantly.

The enzyme may be produced by any method found suitable, e.g. from a native organism, recombinantly in a host organism or the enzymes may also be produced synthetically by e.g. peptide synthesis.

Accordingly, the enzyme having phospholipase activity of the invention may be obtained from a microorganism by use of any suitable technique. For instance, an

enzyme preparation comprising the enzyme having phospholipase activity may be obtained by fermentation of a suitable microorganism capable of producing the enzyme in question and subsequent isolation of a phospholipase enzyme preparation from the resulting fermented broth or microorganism by methods known in the art. The enzyme having phospholipase activity may also be obtained by use of recombinant DNA techniques. Such method normally comprises cultivation of a host cell transformed with a recombinant DNA vector comprising a DNA sequence encoding the enzyme in question and the DNA sequence being operationally linked with an appropriate expression signal such that it is capable of expressing the enzyme having phospholipase activity in a culture medium under conditions permitting the expression of the enzyme and recovering the enzyme from the culture. The DNA sequence may also be incorporated into the genome of the host cell.

The enzyme having phospholipase activity may be in any form suited for the use in question, such as e.g. in the form of a dry powder or granulate, a non-dusting granulate, a liquid, a stabilized liquid, or a protected enzyme. Granulates may be produced, e.g. as disclosed in US 4,106,991 and US 4,661,452, and may optionally be coated by methods known in the art. Liquid enzyme preparations may, for instance, be stabilized by adding stabilizers such as a sugar, a sugar alcohol or another polyol, lactic acid or another organic acid according to established methods. Protected enzymes may be prepared according to the method disclosed in EP 238,216.

The enzyme having phospholipase activity, may be of any origin, e.g. of animal origin (such as, e.g. mammalian), e.g. from pancreas (e.g. bovine or porcine pancreas), or snake venom or bee venom. The enzyme may also be of plant origin. Alternatively, the enzyme having phospholipase activity may be of microbial origin, e.g. from filamentous fungi, yeast or bacteria, such as the genus or species *Thermomyces* (*Humicola*), e.g. *T. lanuginosusl*; *Candida*, e.g *C. antarctica*; *Pseudomonas*, e.g. *P. cepacia; Aspergillus*, e.g. *A. niger*; *Dictyostelium*, e.g. *D. discoideum*; *Mucor*, e.g. *M. javanicus, M. mucedo, M. subtilissimus*; *Neurospora*, e.g. *N. crassa*; *Rhizomucor*, e.g. *R. pusillus or R. miehei*; *Rhizopus*, e.g. *R. arrhizus, R. japonicus, R. stolonifer, R. delemar*; *Sclerotinia*, e.g. *S. libertiana*; *Trichophyton*, e.g. *T. rubrum*; *Whetzelinia*, e.g. *W. sclerotiorum*; *Bacillus*, e.g. *B. megaterium, B. subtilis*; *Citrobacter*, e.g. *C. freundii*; *Enterobacter*, e.g. *E. aerogenes, E. cloacae Edwardsiella, E. tarda*; *Erwinia*, e.g. *E. herbicola*; *Escherichia*, e.g. *E. coli*; *Klebsiella*, e.g. *K. pneumoniae*; *Proteus*, e.g. *P. vulgaris*; *Providencia*, e.g. *P. stuartii*;

*Salmonella*, e.g. *S. typhimurium*; *Serratia*, e.g. *S. liquefasciens*, *S. marcescens*; *Shigella*, e.g. *S. flexneri*; *Streptomyces*, e.g. *S. violeceoruber*; *Yersinia*, e.g. *Y. enterocolitica*. Thus, the enzyme having phospholipase acitivity may be fungal, e.g. from the class *Pyrenomycetes*, such as the genus *Fusarium*, such as a strain of *F. culmorum*, *F. heterosporum*, *F. solani*, or a strain of *F. oxysporum*. The enzyme having phospholipase activity may also be from a filamentous fungus strain within the genus *Aspergillus*, such as a strain of *Aspergillus awamori*, *Aspergillus foetidus*, *Aspergillus japonicus*, *Aspergillus niger* or *Aspergillus oryzae*.

A preferred enzyme having phospholipase activity enzyme is derived or obtained from strain of *Fusarium*, particularly *F. oxysporum*, e.g. from strain DSM 2627 as described in WO 98/26057, in particular as described in claim 36 and SEQ ID NO. 2 of WO 98/26057.

In one embodiment, the enzyme having phospholipase activity is not a native cereal enzyme. In further embodiments, the enzyme having phospholipase activity is not an enzyme present naturally in wheat, i.e. it is not a wheat enzyme having phospholipase activity.

In some embodiments, the enzyme having phospholipase activity is essentially pure, i.e. enzyme protein essentially free from components from the native organism. The enzyme may also, e.g., be substantially pure, i.e. substantially free from other enzyme proteins.

The enzyme having phospholipase activity, may or may not have lipase activity, i.e. activity on triglycerides. Accordingly, in one embodiment of the invention, the enzyme having phospholipase activity has also lipase activity. In other embodiments of the invention, the enzyme having phospholipase activity has essentially no lipase activity.

The term enzyme having phospholipase activity includes whatever auxiliary compounds that may be necessary for the enzyme's catalytic activity, such as, e.g. an appropriate acceptor or cofactor, which may or may not be naturally present in the reaction system.

The enzyme having phospholipase activity may have $A_1$ and/or $A_2$ activity to remove fatty acid from the phospholipid and form a lyso-phospholipid.

Phospholipase A1 may be defined according to standard enzyme EC-classification as EC 3.1.1.32 and for phospholipase A2 as EC 3.1.1.4. The term "phospholipase A" used herein covers an enzyme or enzyme activity with phospholipase A1 and/or phospholipase A2 activity. Also contemplated is treatment

with different types of phospholipase A activities, such as e.g. both of type $A_1$ and $A_2$, or with treatment with only one type of phospholipase activity, such as $A_1$, $A_2$ or B. Thus, in the process of the invention may be used more than one type of enzyme having phospholipase activity.

As disclosed herein, the process of the invention may further comprise addition to the dough of one or more additional enzymes, e.g. but not limited to an amylase and/or a protease. Thus, in addition to the enzyme having phospholipase activity the dough may also be treated with one or more enzyme not having phospholipase activity. These enzymes may be of any origin as disclosed herein mutatis mutandis for the enzyme having phospholipase activity. Accordingly, the additional enzyme used in the process of the invention may be of any origin, including e.g. mammalian, and plant, and microbial (bacterial, yeast or fungal) origin.

**Enzyme treatment**

The enzymatic treatment in the process of the invention may be conducted by incorporating the enzyme into the dough. This may e.g. be done by adding the enzyme into the dough, e.g. by mixing it with the flour and e.g. other dry ingredients and then mixing the flour mix with water or the like and then preparing a dough by mixing.

Thus, the noodle dough comprising the enzyme having phospholipase activity can be manufactured, for example, by adding the enzyme to cereal flours for the production of the noodles, optionally together with other ingredients, e.g. an additional enzyme as described herein, further adding water, and kneading the mixture to form a dough. In other embodiments of the invention the desired ingredients is added to the cereal flours for the manufacture of the noodles, whereas the enzyme is mixed with water and then added to the cereal flour, and kneading the mixture to form a dough. In either case, it is desirable that enzyme(s) is added prior to kneading the dough or at the start of the kneading step, in order for the enzyme to be uniformly dispersed in the dough.

In one embodiment, the process of the invention for producing a fried material, including fried instant noodles, comprises the step of preparing a dough from cereal flour wherein an enzyme having phospholipase activity has been added to the dough, forming the dough into a desired shape, followed by steaming and then frying to obtain a fried cereal product, i.e. the fried starchy product is a fried cereal product.

It is understood that the process is done so as to allow the enzyme reaction to take place at an appropriate holding-time at an appropriate temperature.

The enzymatic treatment may be conducted at any suitable pH, such as e.g. in the range 2-12, such as 2-10, in particular at a pH in the range of 7-12. The process enzymatic treatment may e.g. be conducted at 3-50°C, at a duration found suitable, e.g. for at least 0,1 hours, e.g. in the range of 0,1–6 hours.

The amount of enzyme, may, e.g. be in the range of 0.01-50 mg of enzyme protein per kg of flour, such as e.g. 2-20 mg enzyme protein per kg flour.

The enzyme having phospholipase activity, is added in an amount which provides a desired effect, e.g. an effect as described herein, such as e.g. a decrease in oil uptake during frying of the fried products, e.g. of fried instant noodles. It is understood that the enzyme having phospholipase activity is added to the dough in an effective amount. In one embodiment, the phospholipase activity is added to the flour in an amount of at least 0,5 kLEU per kg flour, such as at least 1 kLEU per kg flour, e.g. in the range of 0,5- 45 kLEU per kg flour, such as e.g. 0,5-20 kLEU per kg flour, e.g. 1 – 20 kLEU per kg flour, or e.g. 5-20 kLEU per kg flour. The Phospholipase activity may e.g. be determined as described in the examples.

It is to be understood that, the enzyme used in the process of the invention, in particular the enzyme having phospholipase activity, is active at a pH range used in the process of the invention. The enzyme is preferably active somewhere in the range of pH 2-12, or at least active in the whole of or part of the range of pH 7-12, and even more preferrebly between pH 8-11.

**Products and uses of the Invention**

As disclosed in the examples, the present invention in one aspect relates to a process as disclosed herein, wherein the fried product(s) manufactured thereby have a reduced oil content compared to a similar process but without use of said enzyme having phospholipase activity. In further aspects, the invention relates to a process of the invention, wherein said fried product is a fried instant noodle having one or more of the properties selected from the group consisting of: increased surface firmness, increased core firmness, improved texture, increased surface smoothness, improved mouthfeel, improved cooked noodle appearance, or reduced oil content compared to a similar process without use of said enzyme.

By the process of the invention, the uptake of oil during frying may be reduced by at least 1%, preferably at least 2%, such as, e.g., in the range of 1-10% compared

to a similar process without the treatment with the enzyme having phospholipase activity. The total oil/fat content of fried instant noodles may be determined by the assay as described in the examples.

The invention also relates to a fried product obtainable or obtained by any of the processes as disclosed herein, including e.g. a fried instant noodle and a snack product produced by the process of the invention. Thus, within the scope of the invention is a snack product or a fried instant noodle obtainable or obtained by any process as described herein.

The kind of fried instant noodles manufactured by the present invention is not limited, examples of which can include Chinese-style fried instant noodles; Japanese-style fried instant noodles such as fried instant ramen, Korean style fried instant noodles such as fried instant ramyun, fried pack noodles, fried cup noodles, fried bowl noodles, and European-style fried instant noodles.

The kind of fried snack product products manufactured by the process of the invention is not limited, examples which include potato chips, corn chips, nachos, and prawn crackers. The process of the invention in addition to the process steps of treating with an enzyme having phospholipase activity and frying, may comprise any additional intermediate process steps suitable for making such fried snack product, e.g. as for snack pellets, also known as 3G products. These practically non-expanded products are cooked and extruded, typically in a single or twin-screw extruder, and are shelf-stable. They are fried at a later stage, typically shortly before being flavoured and packaged by the end manufacturer.

Also included is a process for manufacturing fried snack products wherein snack pellets comprising starch and an enzyme having phospholipase activity are fried. Also included is a process for manufacturing fried snack products wherein snack pellets comprising cereal flour, obtionally isolated starch, and an enzyme having phospholipase activity are fried. The invention further relates to the use of an enzyme having phospholipase activity as described herein in the manufacturing of a fried starchy product, including, e.g. fried instant noodles or fried starchy snack products. The invention also relates to the use of an enzyme having phospholipase activity in a process of the invention as described herein.

The enzyme used in the process of the invention may e.g. be in the form of an enzyme preparation. Thus, the invention also relates to the use of a phospholipase enzyme preparation for the manufacture of a fried product as described herein, e.g.

for obtaining a fried starchy product having a reduced oil content compared to a similar process without use of said enzyme having phospholipase activity.

Accordingly, the invention also relates to the use of an enzyme having phospholipase activity for the manufacturing of fried instant noodles, which when ready to eat have one or more of the properties selected from the group consisting of: increased surface firmness, increased core firmness, improved texture, increased surface smoothness, improved mouthfeel, improved cooked noodle appearance and reduced oil content compared to a process without use of said enzyme. The term "ready to eat" in relation to fried instant noodles is when the fried instant noodles have been rehydrated e.g. by being soaked in very hot water or by being boiled in water for a short period such as in the range 0,5 – 6 min preferrably 1 - 3 min.

In further aspects, the invention also relates to use of an enzyme having phospholipase activity for treating flour, e.g. wheat flour, to be used in a process for making fried instant noodles. Also included are use of an enzyme having phospholipase activity to make a flour mix comprising cereal flour, e.g. wheat flour, and said enzyme, said pre-mix to be used in a process for making fried instant noodles or other fried starchy product such as fried snack products. One embodiment relates to the use of an enzyme having phospholipase activity to make a pre-mix comprising wheat flour, and said enzyme, said pre-mix to be used in a process for making fried instant noodles or other fried starchy product such as fried snack products, wherein said wheat flour has a protein content of less than 15% (w/w), such as less than 12%(w/w), e.g. less than 10%(w/w) before the addition of enzyme. Also provided is use of a phospholipase as described herein for providing a cereal flour mix composition for the manufacture of fried instant noodles, said cereal flour mix composition comprising cereal flours and an enzyme having phospholipase activity.

The present invention is further illustrated in the following examples which is not to be in any way limiting to the scope of protection.

## EXAMPLES

### Texture determinations

*1. Preparation of instant noodles for texture determinations*

Weigh 15g of fried noodle strings. Bring 400 ml tap water to boil in 600 ml beaker. Add the fried noodles and ensure that the water is brought rapidly back to the boil.

Timing is commenced as soon as the noodles are added. Boil the noodles for 2,0 - 5,0 min. Drain the noodles in an appropriate sieve and place them in an iced water bath for 1 min. Then place the cooked noodles in an appropriate tray and leave covered with room temp. tap water for 10 min before testing.

Texture assessments were made using a TA-XT2 Texture Meter (Stable Micro Systems, Goldalming, UK) fitted a 5 kg load cell and a plexiglass "tooth" with a 1 mm flat surface (Oh N.H., Seib P.A., Deyoe, C.W., Ward A.B. 1983 Noodles I. Measuring texture characteristics of cooked noodles. Cereal Chemistry 60: 433 – 438). Texture measurements were made by cutting one boiled noodle strand placed at right angles to the long axis of the plexiglass tooth. The plexiglass tooth was moved downwards at a speed of 1,0 mm/sec to a distance of 0,1 mm from the bottom plate of the texture meter. A primary time-force curve was obtained from the cutting action of the tooth and the parameters surface firmness and core firmness were obtained from this data.

2. *Surface Firmness* is defined as the cutting distance (mm) required to achieve a force value of 0,1 N during the cutting action. In this case, softer noodles require a long cutting distance, compared to harder noodles, to achieve the required force and as such harder noodles have lower numerical values in this definition of surface firmness.

3. *Core firmness* is defined as the maximum force value (N or g) achieved during the cutting stroke. As is more conventional, higher numerical values are indicative of harder noodles in this definition of core firmness.

**Oil/fat content of fried noodle blocks:**

The total oil/fat content of fried instant noodles may be determined by the following assay:

For the purposes of this method fat/oil means the residue obtained after solvent evaporation from a sample treated under the following conditions. In principle the sample is boiled in dilute HCl in order to, among other things, liberate bound fat and convert salts of fatty acids to free acids. After filtration and gentle drying the residue is extracted with petroleum ether or hexane. The solvent is distilled off and the mass of the residue determined.

Fried noodles were ground so as to pass through a 1 mm sieve. An accurately weighed mass of approximately 2,000 g dry matter is added to 50 ml 4 mol/l HCl in a conical flask and the sample boiled gently for 1 h under reflux. Add 1,5 – 3 g filter aid

(Celite, Hyflo or equivalent) in order to avoid fat loss during filtration. Mix and filter sample through moistened filter paper. Rinse flask and condenser with 50 ml warm (50°C) water and pour the water into the filter. Repeat twice. Wash residue with 50 ml portions of cold (< 15°C) water until a neutral pH of filtrate is obtained. Drops of fat on the filtrate surface indicate losses and the determination must be repeated.

Dry the filter paper on a watch glass at 102 ± 2°C for 90 min. Add filter paper and dried residue into a Soxhlet extraction thimble and cover with fat free cotton wool. Place the thimble in the Soxhlet extraction equipment for 6 h. The amount of extraction solvent in the weighed flask should be 1,5 – 2 times the volume of the extraction equipment. During continuous extraction 9 –11 ml of solvent should condence per min.

After completion of extraction distill off the solvent. Dry the flask at 102 ± 2°C for 2 h. allow to cool to room temperature for at least 30 min, protected against dust and moisture e.g. in a dessicator, and weigh to an accuracy of three decimal places. Repeat until differences between successive weightings are less than 0,1% of the weighed amount of sample. The fat content is expressed as a percentage by weight of the dry sample used.

**Phospholipase activity may be determined by the following assay:**

Phospholipase activity (LEU) is measured as the release of free fatty acids from lecithin. 50 µl 4% L-alpha-phosphatidylcholine (plant lecithin from Avanti), 4 % Triton X-100, 5 mM $CaCl_2$ in 50 mM HEPES, pH 7 is added 50 µl enzyme solution diluted to an appropriate concentration in 50 mM HEPES, pH 7. The samples are incubated for 10 min at 30 °C and the reaction stopped at 95 °C for 5 min prior to centrifugation (5 min at 7000 rpm). Free fatty acids are determined using the NEFA C kit from Wako Chemicals GmbH; 25 µl reaction mixture is added 250 µl Reagent A and incubated 10 min at 37 °C. Then 500 µl Reagent B is added and the sample is incubated again, 10 min at 37 °C. The absorption at 550 nm is measured using an HP 8452A diode array spectrophotometer. Samples are run at least in duplicates. Substrate and enzyme blinds (preheated enzyme samples (10 min at 95 °C) + substrate) are included. Oleic acid is used as a fatty acid standard. 1 LEU equals the amount of enzyme capable of releasing 1 µmol of free fatty acid/min at these conditions.

**Example 1 Fried instant noodles - phospholipase treatment**

Method:

An enzyme with phospholipase activity was dissolved in water and added to flour to make a dough for instant noodle production at dosage rates of 0, 4.8, 9.4 and 14.3 mg enzyme protein having phospholipase activity (i.e. the enzyme disclosed in SEQ ID NO. 2 of WO 98/26057) per kg flour.

Dough formulation for noodles with or without enzyme additions was:

- 300 g of wheat flour ("Pelikaan", Meneba Flour Mills, Rotterdam, The Netherlands, protein content 11.4% w/w),
- A solution consisting of 102 g of water, 3 g of NaCl, and 0.9 g of kansui (sodium carbonate).

The ingredients were kneaded into a dough by mixing in a vertical mixer for a total time of 10 min This dough was then compounded (Huo G. (2001) Oriental Noodles. Advances in Food and Nutrition Research Vol 43: 141 – 193) by passing 4 times through noodle-making rollers set at a gap of 3.5 mm. The compounded dough sheet was then rested for 1 h before being reduced in thickness. Thickness reduction was achieved by passing the dough-sheet through sequentially narrower roller gaps of 2.8, 2.2, 1.7, 1.2 and 0.7 mm to obtain a thickness of 1,0 ± 0.1 mm . The sheet was then cut into noodle strands using a rotary cutter to form 1,5 mm wide strands. The resulting raw noodle strands were placed in steaming baskets and steamed at atmospheric pressure at a steam temperature of 100°C for 5 min. The steamed noodles were allowed to cool (0,5 min) and then fried in palm oil at 160°C for 45 s to produced the final steamed and fried instant noodles.

Noodles were assessed for texture and oil content as detailed below and the results are shown in Table 1.

Table 1

| mg enzyme protein/kg flour | Phospholipase activity kLEU/kg flour | **Surface firmness** determined as: penetration distance (mm) to 0,1 N force** | **Core firmness** determined as: maximum cutting force (g)*** | **Oil content (% w/w)** of fried instant noodle blocks |
|---|---|---|---|---|
| 0 | 0 | 0,46 | 34,6 | 20,8 |
| 4,8 | 7,0 | 0,42 | 37,8 | 17,7 |
| 9,4 | 13,7 | 0,40 | 38,9 | 17,9 |
| 14,3 | 20,8 | 0,38 | 39,9 | 18,2 |
| | | ** NB: lower values = harder noodles | ***NB: higher values = harder noodles | |

Result: As can be seen from the results shown in Table 1 addition of the enzyme having phospholipase activity resulted in increased levels of surface firmness which made the noodles more resistant to the eroding effect of the boiling water and less likely to lose mass into the cooking water. These factors combined to give the observed increase in the smoothness of the re-cooked ready-to-eat noodle surfaces. Table 1 also shows that the core of the re-cooked ready-to-eat noodles became more firm after addition of the enzyme having phospholipase activity. The combination of increased surface firmness, the resultant improvement in surface smoothness and the increased core firmness resulted in instant noodles with overall improved textural or mouthfeel characteristics. Cooked noodle appearance was smoother and glossier after cooking. Addition of the enzyme having phospholipase activity also reduced the oil content of the fried instant noodles by up to 3.1% compared to the process without use of said enzyme.

Addition of a 1-3 specific lipase (having the amino acid sequence disclosed in 1-269 of SEQ ID NO: 2 of US 5,869,438) at 30 kLU / kg flour in the same formulation and process as described above for the enzyme having phospolipase activity did not have the same benefits. In particular there were no effects on the oil content of the fried noodles (21,1% compared to 20,8% for the control) or core firmness of the re-cooked ready-to-eat noodles (34,1 g compared to 34,6 g for the control). This suggests that for the enzyme to have these benefits it must contain the phospholipase activity.

The lipase activity (LU) is determined by the following: Lipase activity on tributyrin (LU): A substrate for lipase is prepared by emulsifying tributyrin (glycerin tributyrate) using gum Arabic as emulsifier. The hydrolysis of tributyrin at 30 °C at pH

7 is followed in a pH-stat titration experiment. One unit of lipase activity (1 LU) equals the amount of enzyme capable of releasing 1 µmol butyric acid/min at the standard conditions.

**Example 2 Fried instant noodles - phospholipase treatment**

An enzyme with phospholipase activity was dissolved in water and added to flour to make a dough for instant noodle production at dosage rates of 0, 2.9, 4.3, 8.6 and 12.9 mg enzyme protein having phospholipase activity (i.e. the enzyme disclosed in SEQ ID NO. 2 of WO 98/26057) per kg flour.

- Dough formulation for noodles with or without enzyme additions was: 300 g of wheat flour ("Pelikaan", Meneba Flour Mills, Rotterdam, The Netherlands, protein content 11.4% w/w),
- A solution consisting of 102 g of water, , 3 g of NaCl, and 0.9 g of kansui (sodium carbonate).

Noodles were made and assessed by the method detailed in Example 1.

Noodles were assessed for texture, surface and core firmnesses, and oil content as detailed in example 1 and the results are shown in Table 2

Table 2

| mg enzyme protein/kg flour | Phospholipase activity kLEU/kg flour | Surface firmness determined as: penetration distance (mm) to 0,1 N force** | Core firmness determined as: Maximum cutting force (g)*** | Oil content (% w/w) of fried instant noodle blocks |
|---|---|---|---|---|
| 0 | 0 | 0,34 | 55,9 | 18,1 |
| 2,9 | 4,2 | 0,33 | 61,5 | 16,8 |
| 4,3 | 6,2 | 0,34 | 64,9 | 15,9 |
| 8,6 | 12,5 | 0,34 | 58,9 | 16,4 |
| 12,9 | 18,7 | 0,30 | 66,6 | 16,5 |
| | | ** NB: lower values = harder noodles | ***NB: higher values = harder noodles | |

Result: As can be seen from the results shown in Table 2 addition of the enzyme having phospholipase activity resulted in increased levels of surface firmness at the highest dosage. Observation showed that the boiled noodles also had smoother surface characteristics despite only a marginal change in surface firmness. Table 2 also shows that the core of the noodles became more firm after addition of the enzyme having phospholipase activity. The improvement in surface smoothness and the increased core firmness resulted in instant noodles with overall improved textural or mouthfeel characteristics. Addition of the enzyme having phospholipase activity

also reduced the oil content of the fried instant noodles by up to 3.2% compared to the process without use of said enzyme.

CLAIMS

1. A process for manufacturing a fried product, said process comprising the steps of: (i) incorporation of an enzyme having phospholipase activity into a dough; and (ii) frying to obtain a fried product.

2. The process of claim 1, comprising the step of shaping the dough into a desired form prior to frying.

3. The process of claim 1 or 2, said process comprising the step of steaming prior to frying.

4. The process of any of claims 1-3, wherein the dough is made from flour or semolina from a cereal and/or from another plant source.

5. The process of claim 4, wherein said cereal is selected from the group consisting of, but not limited to, wheat, durum wheat, rye, barley, oats, maize or corn, rice, sorghum, millet, and buckwheat, as well as any mixtures thereof.

6. The process of any of claims 1-5, wherein the dough is made from a cereal flour being substantially only wheat flour, which wheat flower comprises less than 15%, e.g. less than 12%, such as less 10% (w/w) protein, and wherein said fried product is fried instant noodles.

7. The process of claim 5, wherein said other plant source is selected from the group consisting of, but not limited to, potato, sweet potato, yam, taro, tapioca, sago, canna, konjak and beans, such as, e.g., soy bean and mung bean.

8. The process of any of the preceding claims, wherein said dough is a noodle dough and the fried product is fried instant noodle.

9. The process of claim 8, said process comprising the steps of (i) preparing a noodle dough comprising cereal flour, water and an enzyme having phospholipase activity; (ii) making noodle strands from the noodle dough; and (iii) frying the noodles strands; optionally steaming the noodle strands prior to frying, either prior to or after step (ii).

10. A process for manufacturing fried instant noodles wherein noodle strands comprising flour, preferably cereal flour, and an enzyme having phospholipase activity are fried, optionally steaming the noodle strands before frying.

11. A process for manufacturing fried snack products wherein snack pellets comprising starch and/or cereal flour and an enzyme having phospholipase activity are fried.

12. The process of any of claims 9-11, wherein said flour is wheat flour.

13. The process of any preceding claim, wherein the enzyme having phospholipase activity is derived from a microorganism, such as a fungus or a bacterium, preferably from Fusarium, most preferably from F. oxysporum.

14. The process of any preceding claims, wherein said enzyme has phospholipase A activity.

15. The process of any of the preceding claims, wherein said enzyme having phospholipase activity has also lipase activity.

16. The process of any of claims 1-14, wherein said enzyme having phospholipase activity has essentially no lipase activity.

17. The process of any preceding claim, further comprises addition to the dough of one or more additional enzymes selected from the group consisting of an amylase and a protease.

18. The process of any of the preceding claims, wherein the phospholipase activity added to the flour in an amount of at least 0,5 kLEU per kg flour, such as at least 1 kLEU per kg flour, e.g. in the range of 0,5- 45 kLEU per kg flour, such as e.g. 0,5-20 1 – 20 kLEU per kg flour, e.g. 1 – 20 kLEU per kg flour, e.g. 5-20 kLEU per kg flour.

19. The process of any preceding claim, wherein the product is fried instant noodles made from a noodle dough comprising cereal flour, water and said enzyme having phospholipase activity.

20. The process of claim 19, wherein the flour is wheat flour.

21. The process of claim 18 or 20, wherein the dough further comprises one or more ingredients selected from the group consisting of a chemical leavening agent, NaCl, kansui (kansui such as, e.g., but not limited to, sodium carbonate, potassium carbonate or a mixture of these or other salts).

22. The process of any of claims 19-21, wherein the dough further comprises one or more of the ingredients selected from the group consisting of guar gum, locust bean gum, alginates, xanthan gum, carboxymethyl-cellulose, methyl-cellulose, monoglycerides, diacetyltartaric esters of mono-and di-glycerides (DATEM), wheat gluten, phosphate salts, emulsifying agents, protein enhancing agents, polysaccharides for increasing viscosity, oligosaccharides, polymerized phosphates, coloring materials, nutrition enhancing agents, powdered chlorella, powdered skim milk, powdered vegetables, powdered seaweeds, antioxidants, whole egg, egg white and the like, or native starches from botanical sources other than the primary cereal flour used to make the dough, or chemically or physically modified starches from any botanical source which could include one or more starches selected from, but not limited to, the group hydroxypropyl starches or cross-linked starches or partly hydrolyzed starches or cold-water swelling starches.

23. The process of any preceding claims wherein the dough does not comprise a chemical leavening agent.

24. The process of any of the preceding claims, wherein said fried product has a reduced oil content compared to a process without use of said enzyme having phospholipase activity.

25. The process of any of the preceding claims, wherein said fried product is a fried instant noodle having one or more of the properties selected from the group consisting of: increased surface firmness, increased core firmness, improved texture,

increased surface smoothness, improved mouth feel, improved cooked noodle appearance and reduced oil content compared to a process without use of said enzyme.

26. The process of any of claims 1-24, wherein said fried product is a snack product.

27. The process of any of the preceding claims, wherein said fried product is a fried starchy product and wherein said dough is a starchy dough.

28. A fried product obtainable or obtained by a process as defined in any of the preceding claims.

29. A fried instant noodle obtainable or obtained by a process as defined in any of claims 1-25.

30. Use of an enzyme having phospholipase activity in the manufacturing of a fried starchy product, preferably prepared from a dough.

31. Use of a phospholipase enzyme preparation for the manufacture of a fried product prepared from a dough.

32. Use of claim 30 or 31, for obtaining a fried product having a reduced oil content compared to a similar process without use of said enzyme having phospholipase activity.

33. Use of any of claims 30-32, wherein said fried product is a fried instant noodle.

34. Use of an enzyme having phospholipase activity for the manufacturing of fried instant noodles which when ready to eat have one or more of the properties selected from the group consisting of: increased surface firmness, increased core firmness, improved texture, increased surface smoothness, improved mouthfeel, improved cooked noodle appearance and reduced oil content compared to a process without use of said enzyme.

35. Use of an enzyme having phospholipase activity for the manufacturing of fried snack product which when ready to eat have one or more of the properties selected from the group consisting of texture, improved appearance and reduced oil content compared to a process without use of said enzyme.

36. Use of an enzyme having phospholipase activity for treating wheat flour to be used in a process for making fried starchy products, including e.g. fried instant noodles or fried snack products.

37. Use of an enzyme having phospholipase activity to make a pre-mix comprising flour and said enzyme, said pre-mix to be used in a process for making fried products.

38. Use of an enzyme having phospholipase activity to make a pre-mix comprising wheat flour and said enzyme, said pre-mix to be used in a process for making fried instant noodles or fried snack products.

39. Use according to any of claims 36-38, wherein said wheat flour has a protein content of less than 15% (w/w), such as less than 12%, e.g. less than 10%.

40. Use according to any of claims 30-39, wherein the product is produced according to the process as defined in any of claims 1-27.

41. Use according to any of claims 30-40, wherein the enzyme having phospholipase activity is as defined in any of claims 13-16.

**Abstract**

A process for producing fried starchy products, based on e.g. cereal flour. The product may e.g. be fried instant noodles or snack product. The methods comprise treatment with an enzyme having phospholipase activity, e.g. phospholipase A and frying. By the invention is provided a method for decreasing the oil content of the fried product and/or improving texture.